Protein plays matchmaker for QD–antibody conjugation

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Research Profiles
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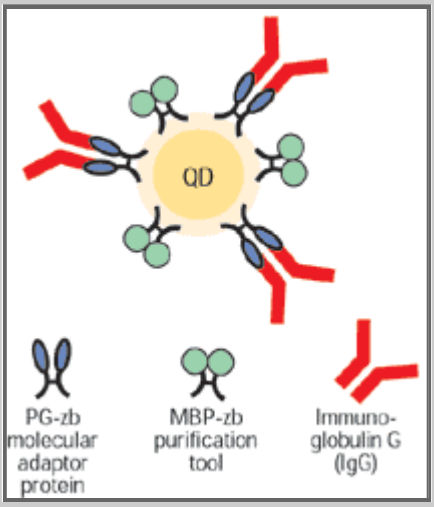
For a team of researchers at the Naval Research Laboratory (NRL) in Washington, D.C., conjugating luminescent quantum dots (QDs) with antibodies became an adventure into perseverance and exciting possibilities.



Cartoon of a mixed-surface composition QD–antibody conjugate.

In the summer of 1999, the group experimented with common covalent chemistry to form QD–antibody bioconjugates but ended up with useless aggregated dots. It was like "running into the brick wall," recalls George Anderson, one of several researchers on the project. "We tried all the conventional covalent techniques over and over and were just kind of frustrated day after day with that."

But the NRL researchers began collaborating with other scientists and came up with an engineered adaptor protein to conjugate the QDs with antibodies. It was a perfect fit. The result is a new kind of fluoroimmunoassay that has the potential to be a powerful tool in medical diagnostics and biowarfare detection, say Ellen Goldman and Hedi Mattoussi. When they realized the fruits of their labor, the research "went from frustrating to fun very quickly," says Anderson.

In the Feb. 15 issue of *Analytical Chemistry* (pp 841–847), Anderson, Goldman, Mattoussi, and colleagues describe how they attached the immunoglobulin G (IgG)-binding ß2 domain of a modified streptococcal protein G (PG) to the highly luminescent semiconductor CdSe–ZnS core-shell QDs via electrostatic self-assembly. The product was a QD–antibody conjugate (QD–IgG) with an emission maximum of 570 nm.

The electrostatic interaction arises from negatively charged dihydrolipoic acid (DHLA)-capped QDs capturing positively charged basic leucine zipper domains appended to the C-termini of PGs. The researchers also developed a purification tool using the maltose binding protein's basic leucine zipper (MBP-zb) on the mixed-surface QDs to remove unbound antibody from the QD–IgG product via affinity chromatography. The scientists can vary the number of antibodies conjugated to a single QD by simply changing the ratio of the molecular adaptor protein, the PG basic leucine zipper (PG-zb), and the MBP-zb that are incubated with the QDs.

They used the QD–IgG conjugates in direct and sandwich fluoroimmunoassays to successfully detect the protein toxin staphylococcal enterotoxin B and the small-molecule explosive 2,4,6-trinitrotoluene. The researchers say their method is different from previously reported conjugation techniques, such as those using avidin–biotin technology or covalent cross-linking. People still use the covalent bond approach, but aggregation remains

an issue with that technique, says Mattoussi. In the NRL strategy, however, which involved help from Moungi Bawendi's group at the Massachusetts Institute of Technology, aggregate-free conjugates worked well in fluoroimmunoassays. "We were really surprised," says Anderson. "One of the first observations was when we mixed the proteins with the dots, there was a great jump in luminescence output."

The most trying moments of the project involved purifying the molecular adaptor protein and making the mixed-surface QDs. While Mattoussi and Anderson were examining the chemistry involved in attaching the antibodies to QDs, Goldman and J. Matthew Mauro had been working on chimeric recombinant proteins with combinations of functional domains, which eventually led to using an adaptor protein for the conjugation. In the beginning, they had a lot of nucleic acid contamination in their adaptor protein preparations. They had to rethink their strategy and eventually solved that issue by using a denaturing preparation, says Goldman. Another hurdle was making the QDs themselves. They had to be water-soluble and compatible for making bioconjugates, says Mattoussi. The mixed-surface QDs were prepared by incubating DHLA-capped QDs with various molar ratios of purified PG-zb dimer mixed with purified *E. coli* MBP containing the C-terminal peptide linker and the positive leucine zipper.

The researchers point out that luminescent QDs as inorganic fluorophores have the potential to circumvent some of the functional limitations encountered by organic dyes in biotechnological applications. They are resistant to photobleaching and can be excited over a continuous range of wavelengths.

The researchers are working on optimizing immunoassay sensitivity using different colored dots to simultaneously look for multiple toxins or small molecules in the same sample, say the researchers. But they would like to make the QDs more stable at lower pHs, which would make them more useful for environmental analysis. Currently, the QDs only work at pH >7.

They'd also like to change the type of surface charge and improve the IR imaging capability of the QDs to make them ideal for use with tissue, says Mattoussi. For the researchers, the future is bright and hopeful. "This is science, so things change," adds Mattoussi. "Maybe in two years, there will be some other ideas that we never thought of."